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Involvement of monoaminergic systems in the antidepressant-like effect of *Eugenia brasiliensis* Lam. (Myrtaceae) in the tail suspension test in mice

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ABSTRACT

Ethnopharmacological relevance: Several species of *Eugenia* L. are used in folk medicine for the treatment of various diseases. *Eugenia brasiliensis* is used for the treatment of inflammatory diseases, whereas *Eugenia. uniflora* is used for the treatment of symptoms related to depression and mood disorders, and is used in Brazil by the Guarani Indians as a tonic stimulant.
Aim of the study: To investigate the antidepressant-like effect of hydroalcoholic extracts of different plant species of genus *Eugenia* and to characterize the participation of the monoaminergic systems in the mechanism of action of the specie that afforded the most prominent antidepressant-like efficacy.
Materials and methods: In the first set of experiments, the effects of hydroalcoholic extracts of *Eugenia beaurepaireana, Eugenia brasiliensis, Eugenia catharinae, Eugenia umbelliflora* and *Eugenia uniflora* and the antidepressant fluoxetine (positive control) administered acutely by p.o. route were evaluated in the tail suspension test (TST) and locomotor activity was assessed in the open-field test in mice. In the second set of experiments, the involvement of the monoaminergic systems in the antidepressant-like activity of *Eugenia brasiliensis* was evaluated by treating mice with several pharmacological agonists and antagonists. The effects of the combined administration of sub-effective doses of *Eugenia brasiliensis* and the antidepressants fluoxetine, imipramine and bupropion were also evaluated.
Results: The administration of the extracts from *Eugenia brasiliensis, Eugenia catharinae* and *Eugenia umbelliflora*, but not *Eugenia beaurepaireana* and *Eugenia uniflora,* exerted a significant antidepressant-like effect, without altering locomotor activity. The behavioral profile was similar to fluoxetine. Pre-treatment of mice with ketanserin, haloperidol, SCH23390, sulpiride, prazosin and yohimbine prevented the reduction of immobility time induced by *Eugenia brasiliensis*. Treatment with sub-effective doses of WAY100635, SKF38393, apomorphine, phenylephrine, but not clonidine, combined with a sub-effective dose of *Eugenia brasiliensis* decreased the immobility time in the TST. Furthermore, the combined administration of sub-effectives doses of *Eugenia brasiliensis* with fluoxetine, imipramine and bupropion produced an antidepressant-like effect.
Conclusions: This study show, for the first time, the antidepressant-like effect of species of the genus *Eugenia*, especially *Eugenia brasiliensis*, whose effects in the TST seem to be mediated by serotoninergic (5-HT_{1A} and 5-HT_2 receptors), noradrenergic (α_1-adrenoceptor) and dopaminergic (dopamine D_1 and D_2 receptors) systems.

1. Introduction

Depression is a common disorder usually associated with substantial symptom severity and disability that is projected by the World Health Organization as the leading cause disease burden for the year 2030 (Kessler et al., 2003; Lépine and Briley, 2011). Many studies reveal important roles for monoaminergic systems in the pathophysiology and treatment of depression (Coppen, 1967; Elhwuegi, 2004; Schildkraut, 1965). However, the treatment of depression with conventional antidepressants (monoamine oxidase

Abbreviations: ANOVA, analysis of variance; DMSO, dimethylsulfoxide; FST, forced swimming test; MAO-A, monoamine oxidase A; SNRIs, serotonin and noradrenaline reuptake inhibitors; SSRI, selective serotonin reuptake inhibitor; 5-HT, serotonin; TST, tail suspension test

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inhibitors, tricyclics, selective serotonin reuptake inhibitors, selective noradrenaline reuptake inhibitors) provides a complete remission just for 50% of the patients (Nestler et al., 2002; Rush et al., 2003) and produces side effects that may reduce the adhesion of patients to treatment (Brunello et al., 2002; MacGillivray et al., 2003; Sleath et al., 2003).

The use of alternative therapies is highly sought by patients with depression, especially the use of herbal medicines (Kessler et al., 2001; Tachil et al., 2007). Thus, developing safe and effective agents from traditional herbs may provide a good way to lessen the side effects as well as to improve the efficacy of conventional treatment (Wang et al., 2008).

The plants of the genus *Eugenia* L. (Myrtaceae) are used in folk medicine for the treatment of various diseases such as arthritis, rheumatism and diabetes (Revilla, 2002). *Eugenia uniflora* L. is used in folk medicine for the treatment of symptoms related to depression and mood disorders, likely nervousness, anxiety and irritation (Alves et al., 2008; Franco and Fontana, 2004; Greinger, 1996; Korbes, 1995), and is used in Brazil by the Guarani Indians as a tonic stimulant (Alonso, 1998). However, there are no studies regarding the possible antidepressant-like action of this or other species of the genus *Eugenia*.

Therefore, the aim of this study was first to investigate the effects of the hydroalcoholic extracts from *Eugenia beaurepaireana* (Kiaersk.) D.Legrand, *Eugenia brasiliensis* Lam., *Eugenia catharinae* O.Berg, *Eugenia umbelliflora* O.Berg, and *Eugenia uniflora* L. in the tail suspension test, a predictive test of antidepressant activity. In a second set of experiments, this study investigated the possible involvement of the monoaminergic systems in the antidepressant-like effect of the hydroalcoholic extract from *Eugenia brasiliensis* in the TST. Furthermore, the effects of the combined administration of sub-effective doses of *Eugenia brasiliensis* and antidepressants from different classes (the serotonin reuptake inhibitor fluoxetine, the tricyclic antidepressant imipramine and the dopamine reuptake inhibitor bupropion) were evaluated.

2. Methods

2.1. Plant material and preparation of the hydroalcoholic extracts

Eugenia beaurepaireana and *Eugenia brasiliensis* were collected in Santo Amaro da Imperatriz, *Eugenia catharinae* on Daniela beach, *Eugenia umbelliflora* and *Eugenia uniflora* in Florianópolis (Santa Catarina State, Brazil). The identification was performed by botanist Dr. Daniel de Barcellos Falkenberg and a voucher specimen was deposited in the Herbarium of the Department of Botany, Federal University of Santa Catarina (UFSC), Brazil (*Eugenia beaurepaireana*—34.675, *Eugenia brasiliensis*—34.674, *Eugenia catharinae*—27.820, *Eugenia umbelliflora*—17.890 and *Eugenia uniflora*—23.152).

The dried and powdered leaves (100 g) of different species of *Eugenia* were extracted three times by maceration with 96% ethanol for 7 day at room temperature. After extraction, the alcoholic extracts were filtered and the procedure was repeated twice. The resulting extracts were dried and concentrated in rotary evaporator under reduced pressure (60 °C), resulting in 8.97 g, 10.82 g, 15.56 g, 8.18 g and 19.20 g of crude extract of *Eugenia beaurepaireana*, *Eugenia brasiliensis*, *Eugenia catharinae*, *Eugenia umbelliflora* and *Eugenia uniflora*, respectively.

2.2. Animals

Swiss mice (35–45 g) of either sex (homogeneously distributed among groups) were maintained at constant room temperature (21–23 °C) with free access to water and food, under a 12:12 h light:dark cycle (lights on at 07:00 h). Mice were allowed to acclimatize to the holding room for 24 h before the behavioral procedure. All manipulations were conducted in the light phase, with each animal used only once ($n=7$–8 animals per group).

All procedures were performed in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals and approved by the Ethics Committee of the Institution. All efforts were made to minimize animals suffering and to reduce the number of animals used in the experiments.

2.3. Drugs and treatment

The hydroalcoholic extracts and fluoxetine (10 mg/kg, p.o., positive control) were dissolved in distilled water with 10% Tween 80 and administered acutely by oral route (p.o.) 60 min before the TST or open-field test. A control group received distilled water with 10% Tween 80 as vehicle.

The following drugs were used: ketanserin, *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridyl) cyclohexanecarboxamide (WAY100635), haloperidol, R-(+)-7-chloro-8-hydroxy-3 methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine hydrochloride (SCH23390), sulpiride, (±)-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine-7,8-diol hydrochloride (SKF38393), apomorphine, prazosin, yohimbine, phenylephrine, clonidine and fluoxetine (all from Sigma Chemical Company, St. Louis,MO, U.S.A.) All drugs were administered by intraperitoneal (i.p.) route, except fluoxetine that was administered by oral route and WAY100534, SCH23390, SKF38393 and phenylephrine that were administered by subcutaneous (s.c.) route. All drugs were administered in a constant volume of 10 ml/kg body weight. Drugs were dissolved in saline, except sulpiride that was diluted in saline with 5% DMSO and fluoxetine that was diluted in distilled water. Control animals received appropriate vehicle.

In the experiments designed to study the time-course effect of the hydroalcoholic extract of *Eugenia brasiliensis* (1 mg/kg, p.o.), the immobility time in the TST was assessed in an independent group of mice, 30, 60 or 120 min after the administration of the extract.

In order to address some of the mechanisms by which the extract of *Eugenia brasiliensis* causes antidepressant-like action in the TST, mice were treated with different pharmacological agents.

A possible contribution of the serotonergic system (5-HT receptor subtypes) in the antidepressant-like effect of the extract of *Eugenia brasiliensis* in the TST was investigated by pre-treating mice with a sub-effective dose of the extract of *Eugenia brasiliensis* (0.1 mg/kg, p.o.) or vehicle. After 30 min they received a sub-effective dose of WAY100635 (0.1 mg/kg, s.c.) or vehicle before being tested in the TST 30 min later. In a separate set of experiments, animals were pre-treated with ketanserin (5 mg/kg, i.p., a preferential 5-HT_{2A} receptor antagonist), or vehicle and after 30 min, they received the extract of *Eugenia brasiliensis* (1 mg/kg, p.o.) or vehicle before being tested in the TST 60 min later (Freitas et al., 2010).

In order to investigate the possible involvement of the noradrenergic system in the antidepressant-like effect of the extract of *Eugenia brasiliensis* in the TST, animals were pre-treated with prazosin (1 mg/kg, i.p., an α_1-adrenoceptor antagonist), yohimbine (1 mg/kg, i.p., an α_2-adrenoceptor antagonist), or vehicle and after 30 min they received the extract of *Eugenia brasiliensis* (1 mg/kg, p.o.) or vehicle before being tested in the TST 60 min later. In a separate set of experiments, in order to investigate a possible synergistic effect between the extract of *Eugenia brasiliensis* and noradrenergic agonists, animals were pre-treated with a sub-effective dose of the extract of *Eugenia brasiliensis* (0.1 mg/kg, p.o.) or vehicle and after 30 min they received a sub-effective dose of phenylephrine (5 mg/kg, s.c., an α_1-adrenoceptor agonist),

clonidine (0.06 mg/kg, i.p., an α_2-adrenoceptor agonist) or vehicle before being tested in the TST 30 min later (Capra et al., 2010; Freitas et al., 2010; Machado et al., 2007).

We also investigate the influence of the dopaminergic system in the antidepressant-like effect of the extract of *Eugenia brasiliensis* in the TST. Mice were pre-treated with haloperidol (0.2 mg/kg, i.p., a nonselective dopaminergic receptor antagonist), SCH23390 (0.05 mg/kg, s.c., a dopamine D_1 receptor antagonist), sulpiride (50 mg/kg, i.p., a dopamine D_2 receptor antagonist) or vehicle, and after 30 min they received the extract of *Eugenia brasiliensis* (1 mg/kg, p.o.) or vehicle and were tested in the TST 60 min later. In order to investigate a possible synergistic effect between the extract of *Eugenia brasiliensis* and dopaminergic agonists, mice were pre-treated with a sub-effective dose of the extract of *Eugenia brasiliensis* (0.1 mg/kg, p.o.) or vehicle and after 30 min they received a sub-effective dose of SKF38393 (0.1 mg/kg, s.c., a dopamine D_1 receptor agonist), apomorphine (0.5 μg/kg, i.p., a dopamine D_2 receptor agonist) or vehicle before being tested in the TST 30 min later (Binfaré et al., 2009, 2010; Capra et al., 2010; Freitas et al., 2010; Machado et al., 2007, 2009).

In another experiment, the effect of the combined administration of a sub-effective dose of the extract of *Eugenia brasiliensis* (0.1 mg/kg) with sub-effective doses of antidepressants fluoxetine (5 mg/kg), imipramine (0.1 mg/kg) and bupropion (1 mg/kg) in the TST or open-field test was investigated. To this end, mice received extract or vehicle and immediately after, the antidepressant or vehicle was administered. Sixty minutes later, the TST or open-field test was carried out.

The administration schedule and the doses of the drugs used were chosen based on experiments previously performed in our laboratory and literature data confirm the efficacy of the above mentioned protocols (Binfaré et al., 2009, 2010; Brocardo et al., 2008; Capra et al., 2010; Dhingra and Valecha, 2007; Freitas et al., 2010; Kaster et al., 2007; Machado et al., 2007; 2009; O'Neill et al., 2001).

2.4. Tail suspension test (TST)

The total duration of immobility induced by tail suspension was measured according to the method previously described (Steru et al., 1985). Mice both acoustically and visually isolated were suspended 50 cm above the floor by adhesive tape placed approximately 1 cm from the tip of the tail. Immobility time was recorded during a 6-min period. Mice were considered immobile only when they hung passively or stay completely motionless. Conventional antidepressants decrease the immobility time in this test (Cunha et al., 2008; Steru et al., 1985).

2.5. Open-field test

To assess the possible effects of the hydroalcoholic extracts from *Eugenia* species on locomotor activity, mice were evaluated in the open-field paradigm as previously described (Rodrigues et al., 1996, 2002). Mice were individually placed in a wooden box ($40 \times 60 \times 50$ cm) with the floor divided into 12 squares. The number of crossing in the squares with the four paws was registered during a period of 6 min. The floor of the open-field apparatus was cleaned with 10% ethanol between tests.

2.6. Statistical analysis

All experimental results are given as the mean ± S.E.M. Comparisons between experimental and control groups were performed by one-way (dose-response curves and time-course curve) or two-way ANOVA (study of the mechanism of action) followed by Newman Keuls test when appropriate. A value of $P < 0.05$ was considered to be significant.

3. Results

3.1. Effect of hydroalcoholic extracts of the species of the genus Eugenia on the immobility time in the TST and locomotor activity in the open-field test

Fig. 1 shows the effects of the acute administration of *Eugenia beaurepaireana*, *Eugenia brasiliensis*, *Eugenia catharinae*, *Eugenia umbelliflora* and *Eugenia uniflora* by p.o. route in the TST in mice. The one-way ANOVA revealed a significant antidepressant-like effect of hydroalcoholic extracts of *Eugenia brasiliensis* (Fig. 1B) and *Eugenia catharinae* (Fig 1C) (1, 10 and 100 mg/kg), and *Eugenia umbelliflora* (Fig. 1D) (10 and 100 mg/kg) in the TST. However, *Eugenia beaurepaireana* and *Eugenia uniflora* administration produced no significant effect in the TST. Since *Eugenia brasiliensis* produced the most significant results with the lowest effective dose ($P < 0.01$ at the dose of 1 mg/kg, p.o.) when compared with the other species tested, we investigate the involvement of the monoaminergic systems in its antidepressant-like effect.

Fig. 2 shows that the administration of the extracts (*Eugenia beaurepaireana*, *Eugenia brasiliensis*, *Eugenia catharinae*, *Eugenia umbelliflora*, and *Eugenia uniflora*) did not significantly alter the locomotor activity of mice in the open-field test.

In order to investigate the interval time that the extract of *Eugenia brasiliensis* achieves the best performance in the TST, a time-response curve was carried out. Fig. 3 illustrates that the extract of this plant (1 mg/kg, p.o.) produced an antidepressant-like effect 60 min after its administration, but this effect was not observed when the TST was carried out 30 or 120 min after the administration of the extract. Considering these results, all experiments were performed using 60 min as the time interval between the administration of the extract and the TST.

3.2. Investigation of possible mechanisms underlying the antidepressant-like effect of Eugenia brasiliensis in the TST

3.2.1. Involvement of the serotonergic system

The results presented in Fig. 4A shows that treatment with WAY100635 (0.1 mg/kg, s.c., a selective 5-HT_{1A} receptor antagonist) was effective to enhance the effect of a sub-effective dose of *Eugenia brasiliensis* (0.1 mg/kg, p.o.) in the TST. Fig. 4B shows that administration of WAY100635 itself or in combination with extract did not affect the locomotor activity in the open-field test.

The pre-treatment of animals with ketanserin (5 mg/kg, i.p., a preferential 5-HT_{2A} receptor antagonist) significantly prevented the reduction of immobility time induced by the administration of an active dose of *Eugenia brasiliensis* (1 mg/kg, p.o.) in the TST (Fig. 4C), without affecting locomotor activity in the open-field test (Fig. 4D).

3.2.2. Involvement of the noradrenergic system

The results presented in Fig. 5A shows that the pre-treatment of mice with prazosin (1 mg/kg, i.p., α_1-adrenoceptor antagonist) was able to reverse the antidepressant-like effect of *Eugenia brasiliensis* (1 mg/kg, p.o.) in the TST, without affecting locomotor activity in the open-field test (Fig. 5B). The pre-treatment of animals with yohimbine (1 mg/kg, i.p., α_2-adrenoceptor antagonist) was also able to prevent the antidepressant-like effect of *Eugenia brasiliensis* (1 mg/kg, p.o.) in the TST (Fig. 5C), without affecting the locomotor activity in the open-field test (Fig. 5D).

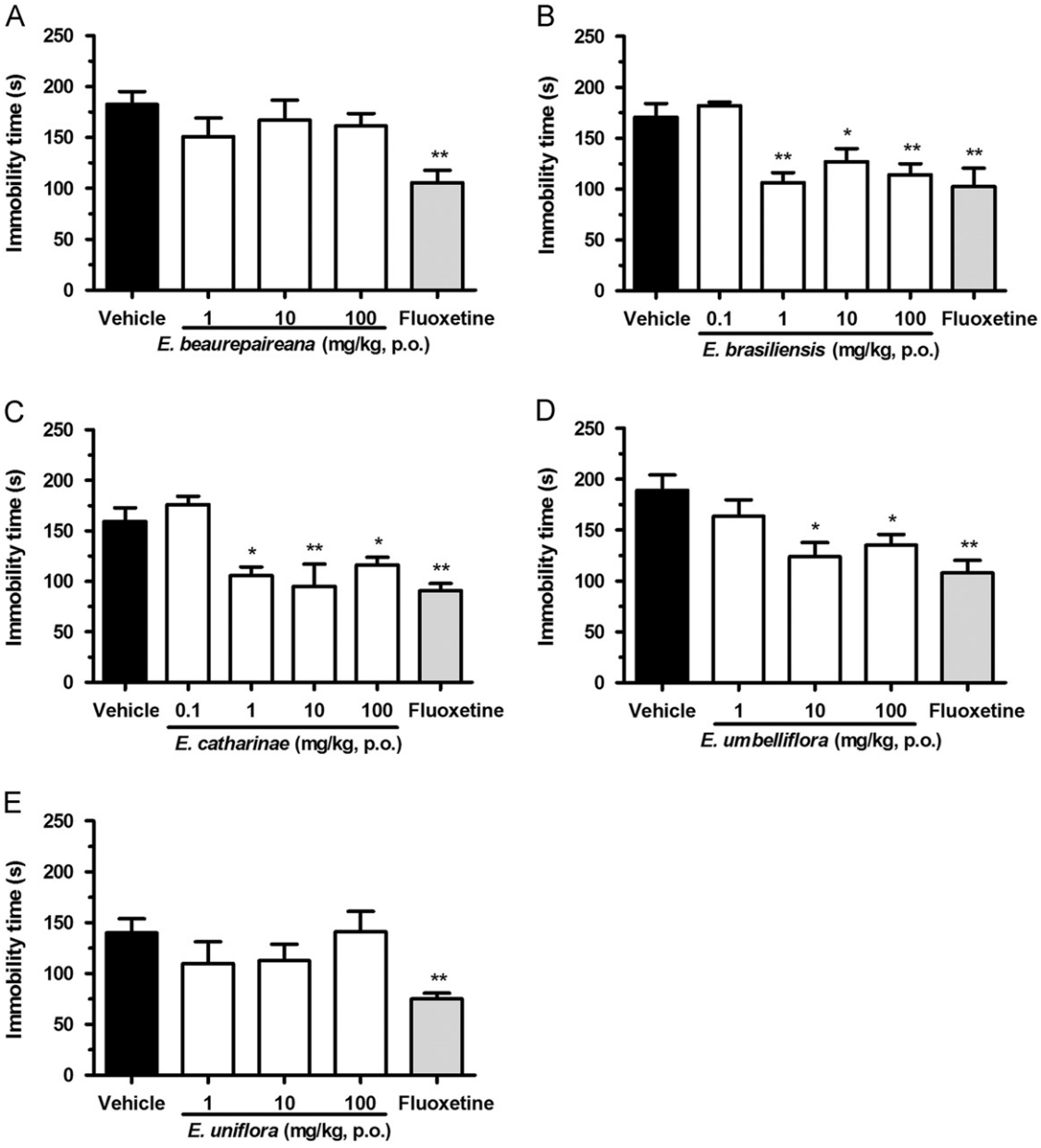

Fig. 1. Effect of treatment with *Eugenia beaurepaireana* (A), *Eugenia brasiliensis* (B), *Eugenia catharinae* (C), *Eugenia umbelliflora* (D) and *Eugenia uniflora* (E) (p.o.), vehicle or fluoxetine in the TST in mice. Values are expressed as mean ± S.E.M (n=7–8). *$P<0.05$ and **$P<0.01$ compared to animals treated with vehicle. (A) [$F(4,35)=3.66$, $P<0.01$]; (B) [$F(5,42)=7.81$, $P<0.01$]; (C) [$F(5,42)=8.26$, $P<0.01$]; (D) [$F(4,35)=5.56$, $P<0.01$]; (E) [$F(4,30)=2.74$, $P<0.05$].

The results of Fig. 6A show that treatment of mice with phenylephrine (5 mg/kg, s.c., α_1-adrenoceptor agonist) potentiated the effect of a sub-effective dose of *Eugenia brasiliensis* (0.1 mg/kg, p.o.) in the TST, without affecting the locomotor activity of animals in the open-field test (Fig. 6B). Fig. 6C shows that the treatment of mice with clonidine (0.06 mg/kg, i.p., α_2-adrenoceptor agonist) was not able to potentiate the effect of a sub-effective dose of *Eugenia brasiliensis* (0.1 mg/kg, p.o.) in the TST and did not modify the locomotor activity in the open-field test (Fig. 6D).

3.2.3. Involvement of the dopaminergic system

The results depicted in Fig. 7A show that the pre-treatment of mice with haloperidol (0.2 mg/kg, i.p., non-selective dopamine receptors antagonist) prevented the reduction of immobility time induced by the treatment of mice with an active dose of *Eugenia brasiliensis* (1 mg/kg, p.o.) in the TST. No effect in the locomotor activity in the open-field test was observed (Fig. 7B). Fig. 7C shows that the pre-treatment of mice with SCH23390 (0.05 mg/kg, s.c., dopamine D_1 receptor antagonist) prevented the reduction of immobility time induced by *Eugenia brasiliensis* (1 mg/kg, p.o.) in the TST, without affecting the locomotor activity of mice. (Fig. 7D). Moreover, Fig. 7E shows that the pre-treatment of mice with sulpiride (50 mg/kg, i.p., dopamine D_2 receptor antagonist) was effective in preventing the antidepressant-like effect of *Eugenia brasiliensis* (1 mg/kg, p.o.) in the TST, but did not alter the locomotor activity in the open-field test (Fig. 7F).

The results presented in Fig. 8A show that treatment of animals with a sub-effective dose of SKF 38393 (0.1 mg/kg, s.c., a dopamine D_1 receptor agonist) potentiated the effect of a sub-effective dose of *Eugenia brasiliensis* (0.1 mg/kg, p.o.) in the TST,

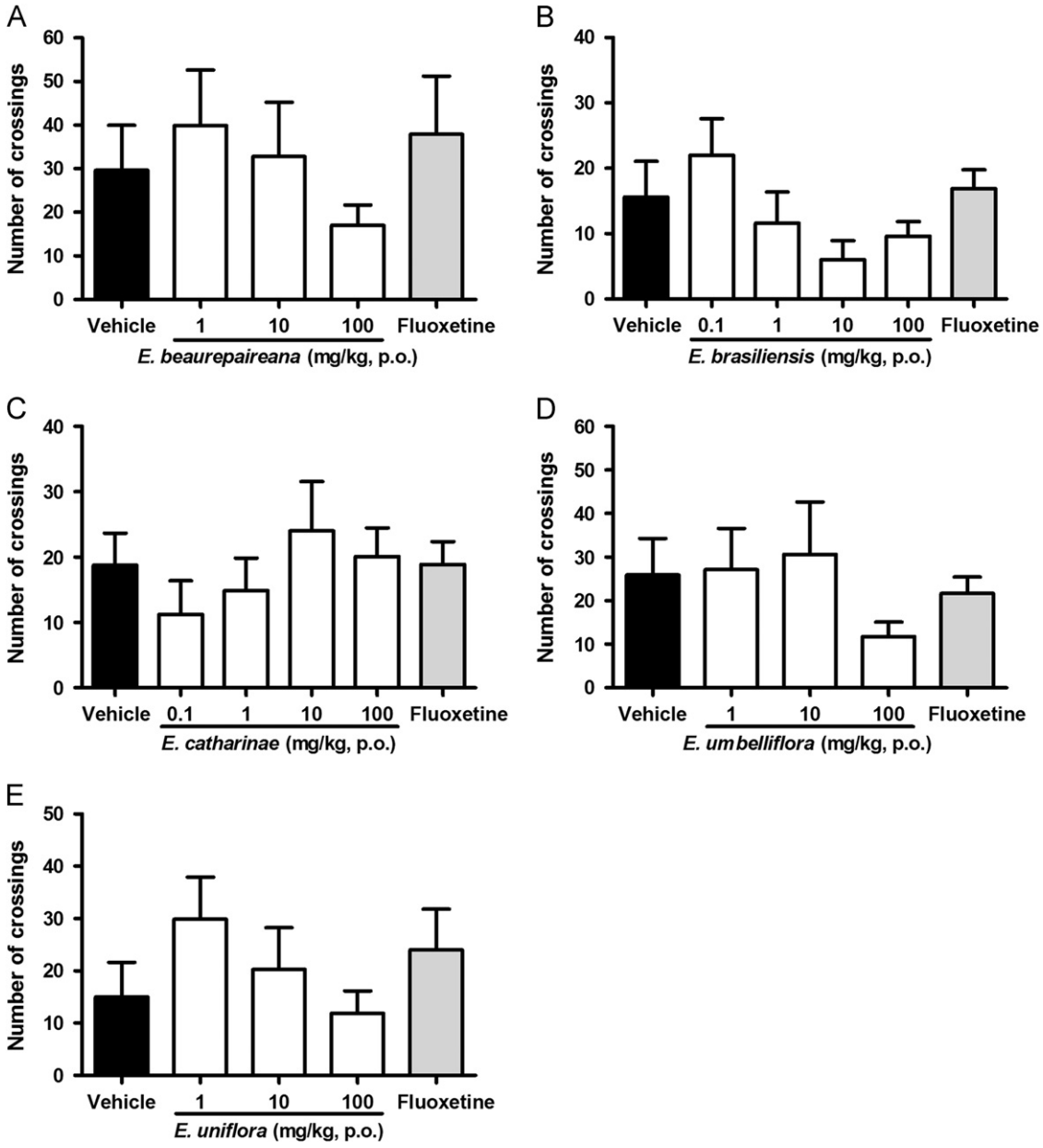

Fig. 2. Effect of treatment with *Eugenia beaurepaireana* (A), *Eugenia brasiliensis* (B), *Eugenia catharinae* (C), *Eugenia umbelliflora* (D) and *Eugenia uniflora* (E) (p.o.), vehicle or fluoxetine in the open-field test. Values are expressed as mean ± S.E.M (n=7–8). (A) [$F(4,35)=0.65$, $P=0.63$]; (B) [$F(5,42)=1.85$, $P=0.12$]; (C) [$F(5,42)=0.71$, $P=0.62$]; (D) [$F(4,35)=0.80$, $P=0.53$]; (E) [$F(4,30)=1.01$, $P=0.42$].

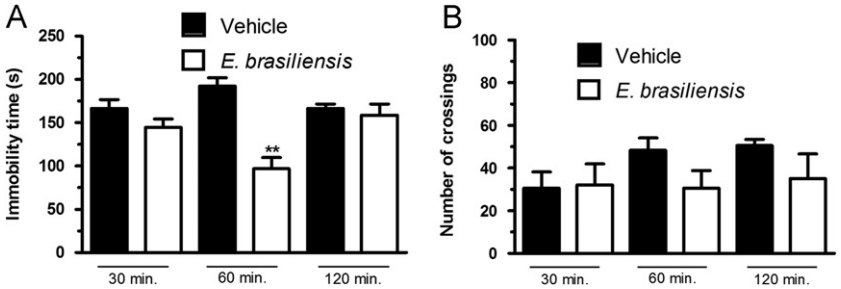

Fig. 3. Effect of an acute administration of the hydroalcoholic extract from *Eugenia brasiliensis* (1 mg/kg, p.o.) 30, 60 or 120 min before the TST (A), and in the open-field test (B). Each column represents the mean ± S.E.M. (n=8). $^{**}P<0.01$ as compared with the vehicle. (A) 30 min ($F(1,14)=2.39$, $P=0.14$]; 60 min [$F(1,14)=35.44$, $P<0.01$]; 120 min [$F(1,14)=0.33$, $P=0.58$]; (B) 30 min [$F(1,14)=0.01$, $P=0.90$]; 60 min [$F(1,14)=3.04$, $P=0.10$]; 120 min [$F(1,14)=1.66$, $P=0.22$].

without affecting the locomotor activity of mice (Fig. 8B). The treatment of animals with a sub-effective dose of apomorphine (0.5 μg/kg, i.p., a dopamine D_2 receptor agonist) potentiated the effect of a sub-effective dose of *Eugenia brasiliensis* (0.1 mg/kg, p.o.) in the TST (Fig. 8C), and did not modify the locomotor activity in the open-field test (Fig. 8D).

3.3. Effect of the interaction of hydroalcoholic extract of Eugenia brasiliensis with conventional antidepressants in the immobility time in the TST, and locomotor activity in the open-field test

The effect of combined administration of a sub-effective dose of the SSRI fluoxetine (5 mg/kg) and *Eugenia brasiliensis* (0.1 mg/kg) on total duration of immobility time in mice are shown in Fig. 9A. This combination significantly decreased the immobility time as compared to vehicle in the TST, without affecting the locomotor activity in the open-field test (Fig. 9B).

The tricyclic antidepressant imipramine administered at a sub-effective dose (0.1 mg/kg, p.o.) produced a synergistic anti-immobility

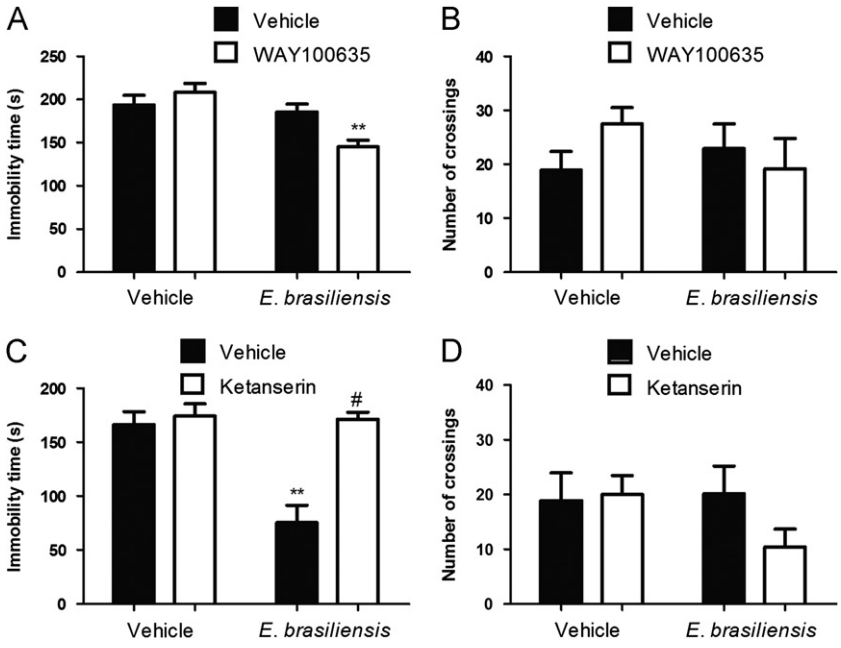

Fig. 4. Effect of the combined treatment with a sub-effective dose of *Eugenia brasiliensis* (0.1 mg/kg, p.o.) and WAY100635 (0.1 mg/kg, s.c.) in the TST (A) and in the open-field test (B). Effect of pre-teatment with ketanserin (5 mg/kg, i.p.) in the effect of *Eugenia brasiliensis* (1 mg/kg, p.o.) in the TST (C), and in the open-field test (D). Each column represents the mean ± S.E.M ($n=8$). $^{**}P<0.01$ compared with the vehicle-treated control. $P<0.01$ as compared with *Eugenia brasiliensis* group pre-treated with vehicle. (A) Pre-treatment [$F(1,28)=13.75$, $P<0.01$], treatment [$F(1,28)=1.88$, $P=0.18$] and pre-treatment x treatment interaction [$F(1,28)=8.18$, $P<0.01$]; (B) Pre-treatment [$F(1,28)=0.26$, $P=0.62$], treatment [$F(1,28)=0.32$, $P=0.58$] and pre-treatment x treatment interaction [$F(1,28)=2.04$, $P=0.16$]; (C) Pre-treatment [$F(1,28)=18.45$, $P<0.01$], treatment [$F(1,28)=15.30$, $P<0.01$] and pre-treatment x treatment interaction [$F(1,28)=13.34$, $P<0.01$]; (D) Pre-treatment [$F(1,28)=1.00$, $P=0.32$], treatment [$F(1,28)=0.94$, $P=0.34$], and pre-treatment x treatment interaction [$F(1,28)=1.59$, $P=0.22$].

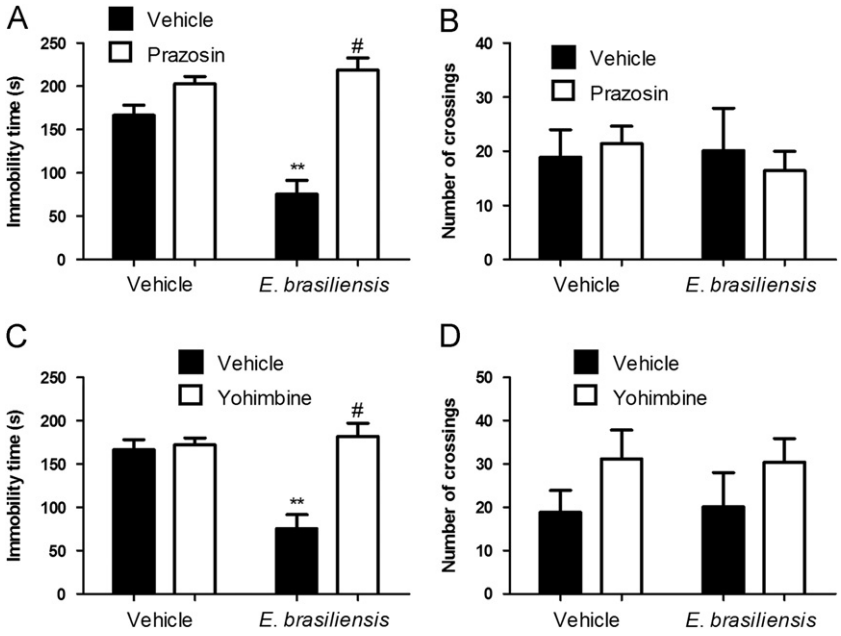

Fig. 5. Effect of pre-teatment with prazosin (1 mg/kg, i.p.) or yohimbine (1 mg/kg, i.p.) in the effect of *Eugenia brasiliensis* (1 mg/kg, p.o.) in the TST (A and C), and in the open-field test (B) and (D). Each column represents the mean ± S.E.M ($n=8$). $^{**}P<0.01$ compared with the vehicle-treated control. $P<0.01$ as compared with *Eugenia brasiliensis* group pre-treated with vehicle. (A) Pre-treatment [$F(1,28)=48.50$, $P<0.01$], treatment [$F(1,28)=8.48$, $P<0.01$] and pre-treatment x treatment interaction [$F(1,28)=17.14$, $P<0.01$]; (B) Pre-treatment [$F(1,28)=0.01$, $P=0.91$], treatment [$F(1,28)=0.13$, $P=0.72$] and pre-treatment x treatment interaction [$F(1,28)=0.35$, $P=0.56$]; (C) Pre-treatment [$F(1,28)=17.98$, $P<0.01$], treatment [$F(1,28)=9.44$, $P<0.01$] and pre-treatment x treatment interaction [$F(1,28)=14.42$, $P<0.01$]; (D) Pre-treatment [$F(1,28)=3.11$, $P=0.09$], treatment [$F(1,28)=0.001$, $P=0.97$] and pre-treatment x treatment interaction [$F(1,28)=0.02$, $P=0.88$].

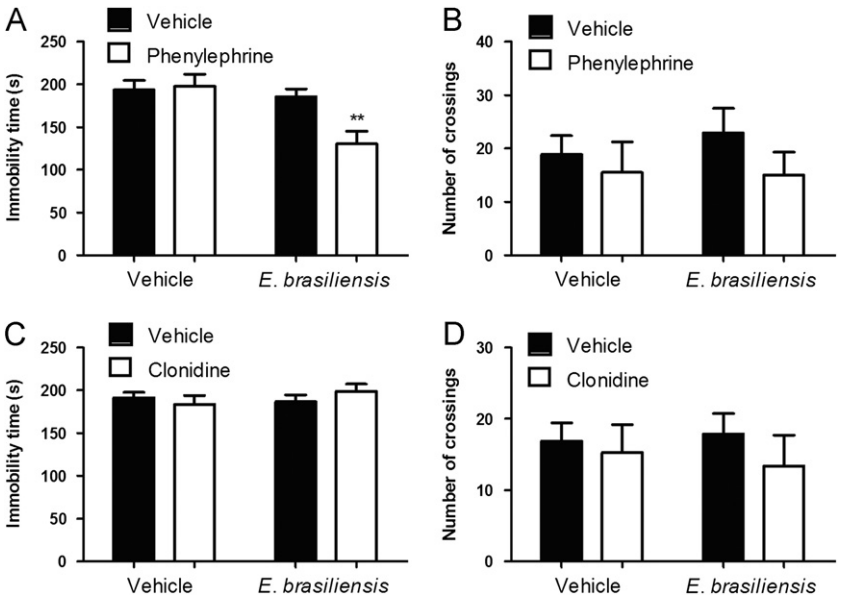

Fig. 6. Effect of the combined treatment with a sub-effective dose of *Eugenia brasiliensis* (0.1 mg/kg, p.o.) and phenylephrine (5 mg/kg, s.c.) or clonidine (0.06 mg/kg, i.p.) in the TST (A and C) and in the open-field test (B) and (D). Each column represents the mean ± S.E.M ($n=8$). $^{**}P<0.01$ compared with *Eugenia brasiliensis* group pre-treated with vehicle. A) Pre-treatment [$F(1,28)=9.37$, $P<0.01$], treatment [$F(1,28)=4.28$, $P<0.05$] and pre-treatment *x* treatment interaction [$F(1,28)=5.78$, $P<0.05$]; (B) Pre-treatment [$F(1,28)=0.14$, $P=0.71$], treatment [$F(1,28)=1.47$, $P=0.23$] and pre-treatment *x* treatment interaction [$F(1,28)=0.24$, $P=0.63$]; (C) Pre-treatment [$F(1,28)=0.42$, $P=0.52$], treatment [$F(1,28)=0.08$, $P=0.78$] and pre-treatment *x* treatment interaction [$F(1,28)=1.31$, $P=0.26$]; (D) Pre-treatment [$F(1,28)=0.02$, $P=0.90$], treatment [$F(1,28)=0.76$, $P=0.39$] and pre-treatment *x* treatment interaction [$F(1,28)=0.17$, $P=0.68$].

effect with a sub-effective dose of *Eugenia brasiliensis* in the TST (Fig. 9C), without altering the locomotor activity in the open-field test (Fig. 9D).

The dopamine reuptake inhibitor bupropion, administered at a sub-effective dose (1 mg/kg), exhibited an antidepressant-like effect when combined with a sub-effective dose of *Eugenia brasiliensis* in the TST (Fig. 9E), without causing alterations in the locomotion in the open-field test (Fig. 9F).

4. Discussion

The TST is a well characterized behavioral model predictive of antidepressant activity. In this test, animals are placed in an inescapable situation and the antidepressant-like activity is expressed by the decrease of immobility time, an effect that is exhibited by conventional antidepressants (Cryan et al., 2005; Steru et al., 1985). However, compounds that promote an increase in locomotor activity of animals can produce a false positive result in the TST. The effects of psychostimulant drugs can be differentiated from antidepressants by the increase in locomotor activity (Borsini et al., 1988). Thus, to rule out the possibility that the reduction in immobility time produced by a given compound in the TST is due to an increase in locomotor activity the animals are submitted to the open-field test (Rodrigues et al., 1996, 2002).

The results of this study show that among the five species analyzed, *Eugenia brasiliensis, Eugenia catharinae* (1, 10 and 100 mg/kg), and *Eugenia umbelliflora* (10 and 100 mg/kg) reduced the immobility time in the TST, without affecting the locomotor activity in the open-field test. Hence, the anti-immobility effect of the extracts in the TST cannot be attributed to a psychostimulant action. *Eugenia uniflora*, despite being used by the Guarani Indians as a tonic stimulant and in folk medicine for the treatment of symptoms related to depression and mood disorders, likely nervousness, anxiety and irritation (Alonso, 1998; Alves et al., 2008; Franco and Fontana, 2004; Greinger, 1996; Korbes, 1995), did not produce a significant antidepressant-like effect in the TST. Moreover, *Eugenia beaurepaireana* also produced no antidepressant-like effects. One possibility that could account for these negative results is that doses higher than those employed in the present study are necessary to cause an effect in the TST. Therefore, more studies are necessary to rule out that *Eugenia uniflora* and *Eugenia beaurepaireana* are devoid of any antidepressant-like effect. In this work, *Eugenia brasiliensis* produced the most significant results in the TST compared with the other species analyzed. Moreover the effective dose (1 mg/kg) can be considered a relatively low dose as compared with effective doses of other plant extracts that show antidepressant-like effects in the TST (Freitas et al., 2010; Lee et al. 2010; Machado et al., 2007, 2009). Therefore, we investigated the possible mechanisms underlying the antidepressant-like action of the extract of *Eugenia brasiliensis* in the TST.

It is important to mention that the chromatographic fractionation of *Eugenia brasiliensis* allowed the isolation of α-amyrin and β-amyrin, betulin acid, 29-hydroxy-oleanolic, and the flavonoids quercetin, catechin and gallocatechin (Magina, 2008). Interesting enough, some of these compounds were reported to cause antidepressant-like effects in behavioral models. Noteworthy, α- and β-amyrin isolated from *Protium heptaphyllum* at doses of 2.5 and 5 mg/kg was shown to exert an antidepressant-like effect in the FST (Aragão et al., 2006). In addition, quercetin also demonstrated an antidepressant-like effect in the FST and exerted a selective inhibitory activity on MAO-A (Bhutada et al., 2010; Chimenti et al., 2006; Kaur et al., 2010; Saaby et al., 2009). Moreover, *in vitro* studies demonstrated that catechin inhibited the uptake of serotonin, norepinephrine and dopamine in cortex, striatum and hippocampal synaptosomes (Rocha et al., 2007). It should also be noted that another flavonoid found in *Eugenia brasiliensis*, rutin, is essential for the antidepressant-like activity of *Hypericum perforatum* in the FST (Fischer et al., 2003; Nöldner and Schötz, 2002). Also, rutin isolated from *Schinus molle* was shown to exert an antidepressant-like effect in the TST (Machado et al., 2008). Therefore, we may suppose that these compounds may be responsible for the antidepressant-like effect obtained when mice were administered with the extract of *Eugenia brasiliensis*.

The monoamines serotonin, norepinephrine and dopamine are involved in the etiology of depression (Elhwuegi, 2004), and the

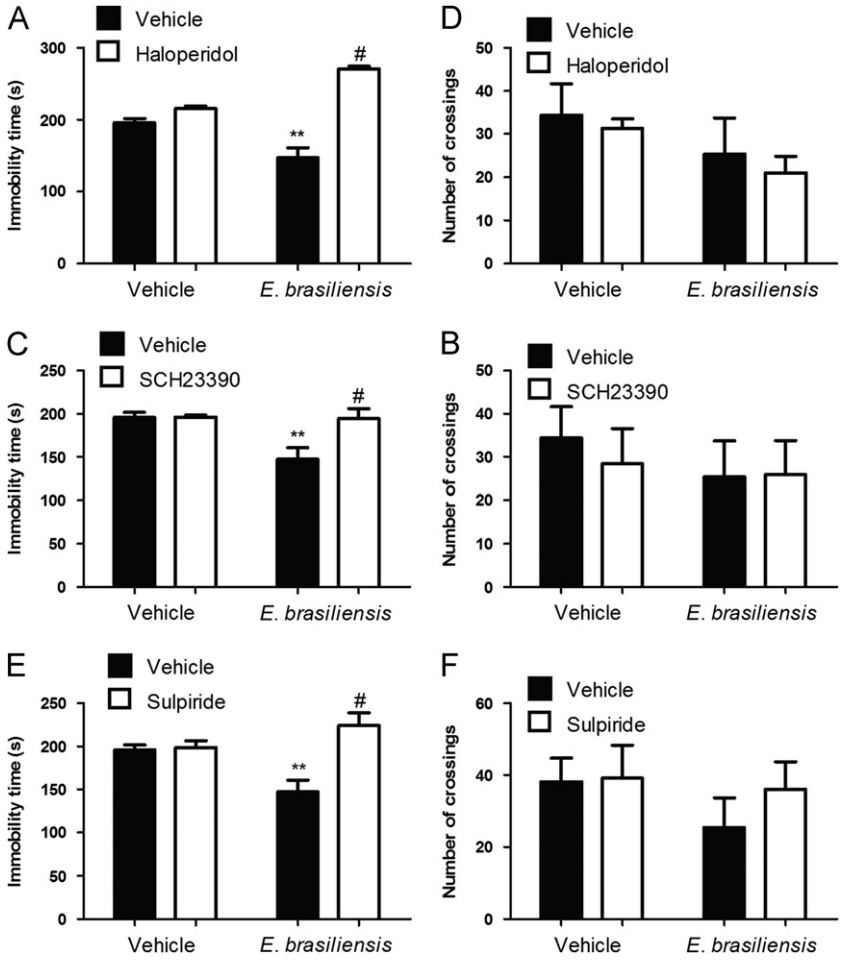

Fig. 7. Effect of pre-treatment with haloperidol (0.2 mg/kg, i.p.), SCH23390 (0.05 mg/kg, s.c.) or sulpiride (50 mg/kg, i.p.) in the effect of *Eugenia brasiliensis* (1 mg/kg, p.o.) in the TST (A, C and E), and in the open-field test (B, D and F). Each column represents the mean ± S.E.M ($n=8$). $^{**}P<0.01$ compared with the vehicle-treated control. $P<0.01$ as compared with *Eugenia brasiliensis* group pre-treated with vehicle. (A) Pre-treatment [$F(1,28)=87.46$, $P<0.01$], treatment [$F(1,28)=0.15$, $P<0.01$] and pre-treatment *x* treatment interaction [$F(1,28)=44.96$, $P<0.01$]; (B) Pre-treatment [$F(1,28)=0.40$, $P=0.53$], treatment [$F(1,28)=2.61$, $P=0.12$] and pre-treatment *x* treatment interaction [$F(1,28)=0.01$, $P=0.92$]; (C) Pre-treatment [$F(1,28)=6.27$, $P<0.05$], treatment [$F(1,28)=6.95$, $P<0.05$] and pre-treatment *x* treatment interaction [$F(1,28)=6.20$, $P<0.05$]; (D) Pre-treatment [$F(1,28)=0.12$, $P=0.73$], treatment [$F(1,28)=0.52$, $P=0.47$] and pre-treatment *x* treatment interaction [$F(1,28)=0.17$, $P=0.68$]; (E) Pre-treatment [$F(1,28)=12.42$, $P<0.01$], treatment [$F(1,28)=1.04$, $P=0.32$] and pre-treatment *x* treatment interaction [$F(1,28)=10.84$, $P<0.01$]; (F) Pre-treatment [$F(1,28)=0.89$, $P=0.35$], treatment [$F(1,28)=0.55$, $P=0.46$] and pre-treatment *x* treatment interaction [$F(1,28)=0.13$, $P=0.72$].

mechanisms of action of conventional antidepressants are based on increasing the bioavailability of monoamines in the brain (Blier, 2001; Brunello et al., 2002). Consistently, the vast majority of drugs currently used to treat depression increase synaptic levels of these monoamines (Risch and Nemeroff, 1992). Moreover, many plant extracts and compounds isolated from these extracts exhibit antidepressant-like effects in the TST, at least partly, by a modulation of the serotonergic system (Capra et al., 2010; Dhingra and Kumar, 2008; Freitas et al., 2010; Machado et al., 2007, 2008; Rodrigues et al., 2002).

The 5-HT_{1A} receptors are directly related to the clinical effects of antidepressants (Celada et al., 2004). These receptors are located presynaptically on the soma and dendrites of 5-HT neurons in the dorsal raphe, act as inhibitory autoreceptors and decrease firing rate and serotonin release (Shrestha et al., 2012). Thus, compounds that block these receptors and prevent this negative feedback might be effective to cause an antidepressant response (Blier and Ward, 2003). The results of the present study show that administration of WAY100635 (5-HT_{1A} receptor antagonist) has a synergistic effect with a sub-effective dose of *Eugenia brasiliensis* (0.1 mg/kg), without affecting the locomotor activity in the open-field test. This result suggests that blocking these receptors, increasing synaptic 5-HT levels, contributes to the antidepressant-like effect of this extract. This result is in line with the finding that the treatment with WAY100635 was able to potentiate the effect of a sub-effective dose of fluoxetine in the FST (Da Rocha et al., 1997) as well as the extract from *Tabebuia avellanedae* (Freitas et al., 2010) in the TST.

Further reinforcing the hypothesis that the antidepressant-like effect of the extract of *Eugenia brasiliensis* is dependent on the serotonergic system, the pre-treatment of mice with ketanserin, a preferential 5-HT_{2A} receptor antagonist, significantly prevented the reduction of immobility time induced by treatment of animals with an effective dose of *Eugenia brasiliensis* (1 mg/kg, p.o.) in the TST, which indicates that the effect of the extract is dependent on an interaction with these receptors. Indeed, literature data show that ketanserin is effective in reversing the antidepressant-like effect of different compounds and extracts as agmatine (Zomkowski et al., 2004) and lectin from *Canavalia brasiliensis* (Barauna et al., 2006) in the FST and *Tabebuia avellanedae*, *Schinus mole* and *Rosmarinus officinalis* in the TST (Freitas et al., 2010; Machado et al., 2007, 2009).

The 5-HT_2 receptors are widely distributed throughout the brain, being present in regions like the prefrontal cortex and hippocampus, which are closely related to the etiology of depression (Ishihara and Sasa, 2001; Krishnan et al., 1991; Nestler et al., 2002). It is suggested

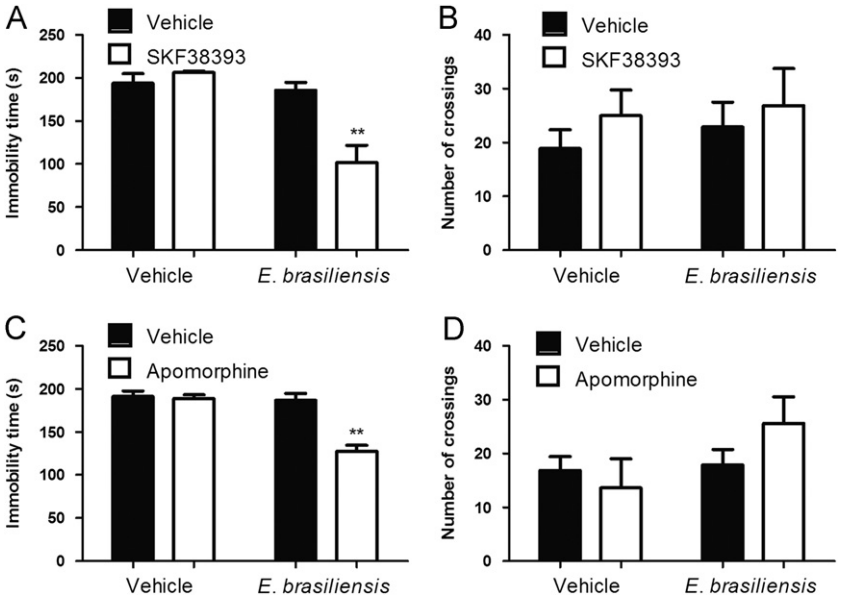

Fig. 8. Effect of the combined treatment with a sub-effective dose of *Eugenia brasiliensis* (0.1 mg/kg, p.o.) and SKF38393 (0.1 mg/kg, s.c.) or apomorphine (0.5 μg/kg, i.p.) in the TST (A) and (C) and in the open-field test (B) and (D). Each column represents the mean ± S.E.M ($n=8$). $^{**}P<0.01$ compared with *Eugenia brasiliensis* group pre-treated with vehicle. (A) Pre-treatment [$F(1,28)=21.38$, $P<0.01$], treatment [$F(1,28)=8.68$, $P<0.01$] and pre-treatment *x* treatment interaction [$F(1,28)=15.64$, $P<0.01$]; (B) Pre-treatment [$F(1,28)=0.33$, $P=0.57$], treatment [$F(1,28)=0.99$, $P=0.33$] and pre-treatment *x* treatment interaction [$F(1,28)=0.04$, $P=0.84$]; (C) Pre-treatment [$F(1,28)=25.03$, $P<0.01$], treatment [$F(1,28)=22.06$, $P<0.01$] and pre-treatment *x* treatment interaction [$F(1,28)=19.11$, $P<0.01$]; (D) Pre-treatment [$F(1,28)=2.48$, $P=0.13$], treatment [$F(1,28)=0.30$, $P=0.59$] and pre-treatment *x* treatment interaction [$F(1,28)=1.77$, $P=0.19$].

that the activation of these receptors may be implicated in the regulation of mood disorders (Celada et al., 2004; Hoyer et al., 1986). In addition, it was shown that DOI, a preferential 5-HT_{2A} receptor agonist increases the effect of several antidepressant compounds (Khisti and Chopde, 2000; Zomkowski et al., 2004).

To reinforce the idea of the involvement of serotonin in the antidepressant-like effect of the extract of *Eugenia brasiliensis*, a possible synergistic effect between the extract and fluoxetine (SSRI) in the TST was investigated. Fluoxetine, an antidepressant that increases the availability of serotonin in the synaptic cleft, when co-administered (p.o.) with the extract of *Eugenia brasiliensis* at sub-effective doses, potentiated the antidepressant-like effect of the extract without causing alterations in the locomotion of mice in the open-field test. Thus, the antidepressant-like effect of the extract appears to be dependent, at least in part, on the increased levels of serotonin in the synaptic cleft.

The noradrenergic system is classically implicated in the pathophysiology of depression and the mechanism of action of antidepressants (Frazer, 2000; Nutt, 2006). Compounds or drugs that affect noradrenergic neurotransmission, such as noradrenaline reuptake inhibitors (SNRIs) or monoamine oxidase inhibitors are used to treat depression (Páez-Pereda, 2005). Preclinical studies demonstrate that the α_1 and α_2-adrenoceptors are involved in the antidepressant-like action of drugs or compounds in animal models of depression (Danysz et al., 1986; Kitada et al., 1983; Masuda et al., 2001). It was observed that the antidepressant-like effect of desipramine (a tricyclic antidepressant with a higher affinity for the reuptake of norepinephrine) was prevented by pre-treatment of mice with prazosin (an α_1-adrenoceptor antagonist) (Danysz et al., 1986). Furthermore, another study demonstrated that the antidepressant-like effect of clonidine (an α_2-adrenoceptor agonist) in the FST was abolished by the pre-treatment with yohimbine, an α_2-adrenoceptor antagonist (O'Neill et al., 2001).

The results of the present study show that pre-treatment with prazosin (an α_1-adrenoceptor antagonist) was able to prevent the anti-immobility effect of *Eugenia brasiliensis* in the TST. Indeed, studies have shown that activation of these receptors is essential for the effect of antidepressants that enhance noradrenergic transmission (Brunello et al., 2002; Millan, 2004). In line with this hypothesis, our results showed that the administration of sub-effective doses of phenylephrine (an α_1-adrenoceptor agonist) with *Eugenia brasiliensis* produced a decrease in the immobility time.

The pre-treatment of mice with yohimbine (an α_2-adrenoceptor antagonist) was also able to prevent the anti-immobility effect of *Eugenia brasiliensis*, suggesting that α_2-adrenoceptors are implicated in the anti-immobility effect of the extract in the TST. However, the administration of a sub-effective dose of clonidine (an α_2-adrenoceptor agonist) in combination with *Eugenia brasiliensis* was unable to reduce the immobility time in the TST. This result do not confirm the involvement of α_2-adrenoceptors in the antidepressant-like effect of *Eugenia brasiliensis*. Considering that yohimbine (1 mg/kg) may act nonselectively as a 5-HT receptor antagonist (Dwoskin et al., 1988), one plausible explanation for our results is that yohimbine abolished the antidepressant-like effect of *Eugenia brasiliensis* by acting at 5-HT receptors, and not directly by the antagonism of α_2-adrenoceptors.

Reinforcing the involvement of serotonergic and noradrenergic systems in the antidepressant-like effect of the extract of *Eugenia brasiliensis*, our study shows that imipramine, administered in combination with the extract of *Eugenia brasiliensis*, at doses that have no effect *per se*, caused a synergistic antidepressant-like effect. Our data indicate that this effect was not due to any locomotor alteration.

Besides the serotonergic and noradrenergic systems, the dopaminergic system is also implicated in mood regulation (D'Aquila et al., 2000; Willner et al., 2005). Several evidence have indicated that antidepressants with effects on the dopaminergic system are effective for the treatment of depression (Papakostas, 2006). Some studies have demonstrated the role of dopamine D_1 and D_2 receptors in depression, since the antagonism of these receptors prevents the effect of some agents with antidepressant-like action in the FST and TST (Borsini et al., 1988; Hirano et al., 2007; Machado et al., 2007; Yamada et al., 2004). Indeed, clinical studies demonstrated that dopamine D_2 receptor agonists are effective

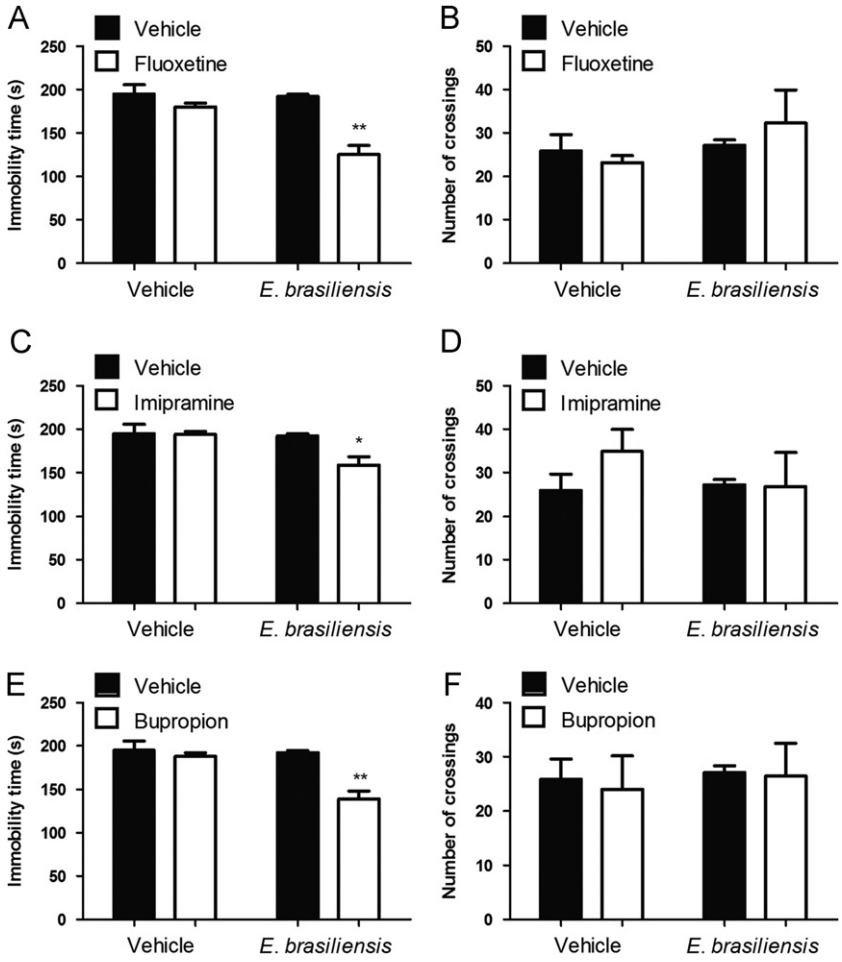

Fig. 9. Effect of the administration of a sub-effective dose of the hydroalcoholic extract from *Eugenia brasiliensis* (0.1 mg/kg, p.o.) and sub-effective doses of fluoxetine (5 mg/kg, p.o.), imipramine (0.1 mg/kg, p.o) and bupropion (1 mg/kg, p.o.) in the TST (A, C and E) and in the open-field test (B), (D) and (F). Each column represents the mean ± S.E.M ($n=8$).*$P<0.05$ and **$P<0.01$ as compared with the vehicle-treated group. (A) Pre-treatment [$F(1,28)=13.25$, $P<0.01$], treatment [$F(1,28)=26.56$, $P<0.01$] and pre-treatment *x* treatment interaction [$F(1,28)=10.75$, $P<0.01$]; (B) Pre-treatment [$F(1,28)=1.39$, $P=0.25$], treatment [$F(1,28)=0.07$, $P=0.79$] and pre-treatment *x* treatment interaction [$F(1,28)=0.80$, $P=0.38$]; (C) Pre-treatment [$F(1,28)=6.42$, $P<0.05$], treatment [$F(1,28)=5.08$, $P<0.05$] and pre-treatment *x* treatment interaction [$F(1,28)=4.65$, $P<0.05$]; (D) Pre-treatment [$F(1,28)=0.46$, $P=0.50$], treatment [$F(1,28)=0.72$, $P=0.40$] and pre-treatment *x* treatment interaction [$F(1,28)=0.85$, $P=0.36$]; (E) Pre-treatment [$F(1,28)=11.63$, $P<0.01$], treatment [$F(1,28)=15.62$, $P<0.01$] and pre-treatment *x* treatment interaction [$F(1,28)=9.19$, $P<0.01$]; (F) Pre-treatment [$F(1,28)=0.14$, $P=0.71$], treatment [$F(1,28)=0.07$; $P=0.79$] and pre-treatment *x* treatment interaction [$F(1,28)=0.02$, $P=0.90$].

for the treatment of patients with depression (Waehrens and Gerlach, 1981). The results of our study show that the dopaminergic system is involved in the antidepressant-like effect of *Eugenia brasiliensis*, since the pre-treatment of animals with haloperidol (a nonselective dopaminergic receptor antagonist), SCH23390 (a dopamine D_1 receptor antagonist) and sulpiride (a dopamine D_2 receptor antagonist) prevented the antidepressant-like effect caused by administration of *Eugenia brasiliensis* in the TST. Moreover, reinforcing the participation of the dopaminergic system in the antidepressant-like effect of *Eugenia brasiliensis*, the treatment with sub-effective doses of SKF38393 (0.1 mg/kg, s.c., a dopamine D_1 receptor agonist) or apomorphine (0.5 μg/kg, i.p., a dopamine D_2 receptor agonist) with *Eugenia brasiliensis* was able to produce an antidepressant-like effect. Interestingly, bupropion, an atypical antidepressant that is a potent inhibitor of dopamine uptake, when co-administered (p.o.) with the extract of *Eugenia brasiliensis*, at doses that have no effect *per se*, potentiates the antidepressant-like effect of the extract, without affecting the locomotor activity of animals.

Considering that treatment of depression with conventional antidepressants produces several side effects (Brunello et al., 2002) that may reduce the adhesion of patients to treatment (MacGillivray et al., 2003; Sleath et al., 2003), there is a need to develop strategies for antidepressant treatment with fewer side effects. Therefore, the results obtained with the co-administration of hydroalcoholic extract of *Eugenia brasiliensis* with the antidepressants fluoxetine, imipramine and bupropion suggest that this extract could be helpful for the improvement of the conventional pharmacotherapy (decreasing the doses of antidepressants prescribed and consequently the side effects). Further studies are necessary to confirm this hypothesis.

5. Conclusions

The present study demonstrated that *Eugenia brasiliensis*, *Eugenia catharinae* and *Eugenia umbelliflora* exert an antidepressant-like effect in the TST. Our findings indicate that the hydroalcoholic extract of *Eugenia brasiliensis* has this effect mediated by serotonergic, noradrenergic and dopaminergic systems. Furthermore, this extract can produce a synergistic antidepressant-like effect with the antidepressants fluoxetine, imipramine and bupropion, suggesting that this extract may improve the effectiveness of these antidepressants. Altogether, the results suggest that *Eugenia brasiliensis* may have potential therapeutic value for the management of depression.

Conflict of interest

The Authors declare that they have no conflicts of interest to disclose.

Acknowledgments

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